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OCCURRENCE OF YEASTS IN YOGHURT, CHEESE AND WHEY

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ABSTRACT 252 yeast strains were isolated from yoghurt (108 strains), soft cheese (50 strains , whey (62 strains) and dairy laboratory (32 strains) On the basis of 25 physiological and morpho logical characteristics, the isolated yeast strains were assigned to 10 species belonging to 9 gen era. *Kluyveromyces marxianus* was the most common species followed by *Debaromyces hansenii* These species were of high occurrence in yoghurt cheese and whey *Rhodotorula mucilaginosa, Saccharomyces cerevisiae* and *Sporobolomyces roseus* were represented by a considerable number of strains The total counts of yeast cells ranged between 9 8 x 10^3 and 1 8 x 10^5 in yoghurt, 1 1 x $.0^4$ and 6 4 x 10^4 in cheese and 4 0 x 10^4 and 1 1 x 10^5 in whey Our results showed that the composition of yeast flora as well as the total counts of yeast cells were dependent on manufac toring plants and contamination sources Of particular note was the ability of yeast species to grow in yoghurt at preservative temperature (5 6°C) The total counts of yeast cells were obvi ously higher at the surface of yoghurt packings than at the bottom These counts increased three fold at the bottom and ca five fold at the surface of yoghurt packings after incubation of yog hurt packings at 5-6°C for 3 days from manufacturing date It was of interest that *Rhodotorula mucilaginosa, Trichosporon beigelii* and *Candida rugosa*, the human yeast pathogens, as well as *Candida steatolytica*, the erreger of mastitic udder of cow, were isolated in this study

RÉSUMÉ 252 souches de levures ont été isolées de yaourts (108 souches), de fromage mollet (50 souches), de petit lait (62 souches) et de de laboratoires laitiers (32 souches) Sur la base de 25 caractéristiques morphologiques et physiologiques, les souches isolées ont été réparties en 10 espèces appartenant à 9 genres *Kluyveromyces marxianus* est l'espèce la plus commune, suivie par *Debaromyces hansenii* On trouve ces espèces en grande quantité dans le yaourt, le fromage et le petit lait *Rhodotorula mucilaginosa Saccharomyces cerevisiae* et *Sporobolomyces roseus* sont également représentées par un nombre considérable de souches Le nombre total de cellules de levures est compris entre 9,8 10^3 et 1,8 10^5 pour le yaourt, entre 1,1 10^4 et 6,4 10^4 pour le fro mage et entre 4,0 10^4 et 1,1 10^5 pour le petit lait Nos résultats montrent que les espèces et le nombre de cellules de levures isolées dépendent des techniques de fabrication et des sources de contamination On remarquera l'aptitude des espèces isolées à se développer dans le yaourt, aux températures de conservation (5-6°C) On peut observer que le nombre global de cellules est plus important à la surface qu'au fond du pot de yaourt Durant les trois jours suivant la fabrication, à la température de conservation, le nombre de cellules de levure est multiplié par 3 au fond du pot, alors qu'il est multiplié par 5 à la surface du yaourt Cette étude a également permis d'iso ler de levures pathogènes pour l'homme, *Rhodotorula mucilaginosa, Trichosporon beigelii, Candida rugosa*, ou pour l'animal, *Candida steatolytica* cause de la maladie du pis de la vache

KEY WORDS : yeast, yoghurt, cheese, whey, human and animal pathogens

Yeasts represent an obvious group of the microflora of milk and milk products (Dombrowski, 1910) Depending upon type of yeast species as well as degree of product contamination, they affect products positively or negatively. Their positive effects were represented by deassimilation of acids and production of growth factors which stimulate growth and activity of surface flora especially lactic acid bacteria as well as aroma production by the formation of volatile acids and carbonyl compounds (Hosono & Tokita, 1970, Kang et al., 1976, Engel, 1980, Schmidt, 1982, Winkelmann, 1983, Lenoir, 1984) Their negative effects were a result of the fermentation of lactose producing gas which in turn leads to swelling, flawing and bombage of product container (Stocker, 1954, Fahmy & Youssef, 1974, Kroger, 1976, Spillmann & Geiges, 1983), proteolytic and lipolytic activities (Schmidt, 1982) and production of disagreeable flavour, bitter taste and loss of texture quality (Soulides, 1955, Davis, 1970, 1974, Kroger, 1976). In Egypt, yoghurt and and soft cheese represent an important protein source for farmers and the average class population which need to compensate for the amino acids deficiencies of a vegetable protein diet The study of sanitary condition of these milk product is still under way, especially in villages Except for the work done on cheese by Ghoniem (1963, 1968), Fahmy & Youssef (1974), El Bassiony et al (1980), Seham et al (1982) and El Essawy et al (1984), little is known about yeast flora of soft cheese especially at the El-Minia locality, where no study was performed along that line Moreover there is an obvious shortage in the knowledge about yeasts of yoghurt as well as whey Therefore, we report on the yeast flora of these milk products

MATERIALS AND METHODS

Cheese and whey were collected in sterile conical flasks from supermarkets as well as from farmer sellers in El-Minia markets Yoghurt packings of three different producers were bought from super markets. 5-10g samples from cheese or yoghurt were mixed with 50 ml sterile Ringer solution (Haridy, 1987), blended and decimal dilutions were prepared for counting purposes 0 5ml whey samples were mixed with 9 5 ml sterile Ringer solution and series of dilutions were also prepared 0 5ml portions were spread on plates containing yeast malt agar medium (Lodder, 1970) adjusted to pH 3 5 Plates were incubated at 28°C for 3-5 days Developing yeast colonies were counted and after microscopic examination, 252 strains were isolated, purified and preserved on yeast malt agar slants at 4°C In order to survey yeast flora of the dairy laboratory, swabs were made from the floor and equipment and then streaked on the above mentioned plates, whereas air of the dairy laboratory was surveyed by exposing prepared plates to the air In order to test the ability of yeasts to grow in yoghurt at preservative temperature (5 6°C), a set of yoghurt packings were bought from the dairy laboratory after manufacturing and then incubated at 5 6°C Following the above mentioned procedures, the total count of yeasts cells were determined daily for a period of 6 days Identification of the isolated yeast strains was performed on the basis of 25 physiological and morphological properties (table 3) as indicated by Lodder (1970), Barnett et al. (1983) and Kreger van Rij (1984).

RESULTS AND DISCUSSION

I - Yoghurt:

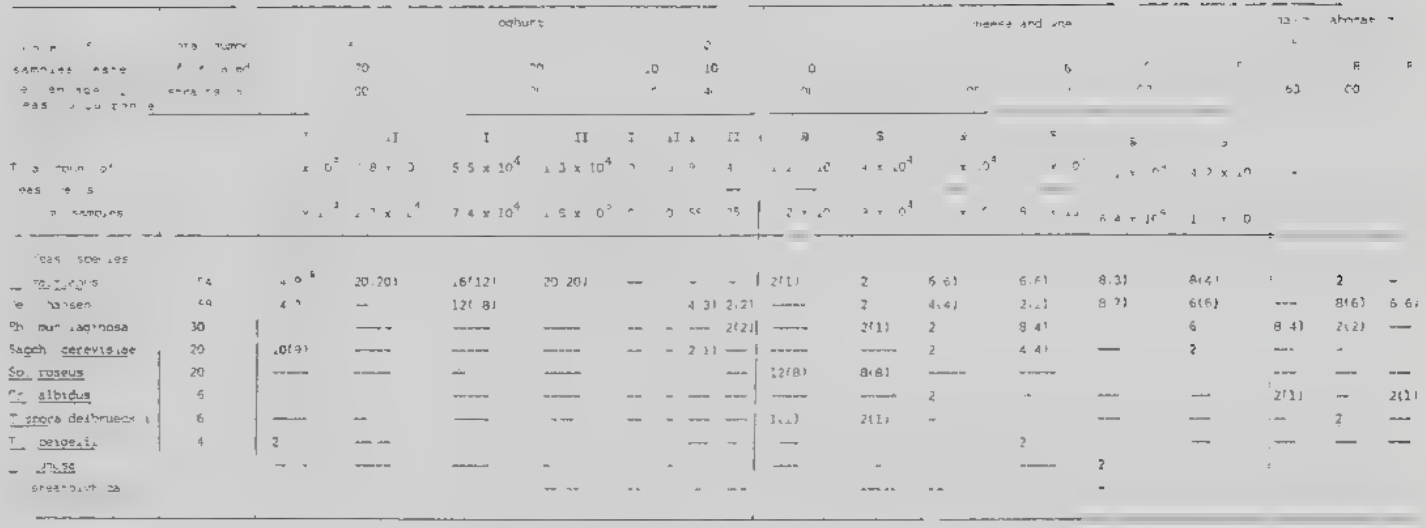
Yoghurt represents a selective environment for the growth of yeasts because of its low pH value Spoilage of yoghurt was reported by several investigators (Ingram,

1958, Walker & Ayres, 1970, Davis, 1975, Peppler, 1976, Spillmann & Geiges, 1983, Hardy, 1987) Results of our investigations of natural yoghurt products from 3 different producers (A, B, and C) showed that natural yoghurt from producer C were free from yeasts, whereas 40% of his fruit yoghurt products (D) contained yeasts (table 1) The total count of yeast cells ranged between 4 and 55 yeast cells per g fruit yoghurt Introduction of fruits into his yoghurt products has amplified the risk of spoilage by yeast by providing additional sources of contamination and fermentable substrates *Debaromyces hansenii* was the most common species These observations were also reported by other investigators (Davis, 1970, 1975, Tilbury et al , 1974; Spillmann & Geiges, 1983, Hardy, 1987) Natural yoghurt of producer B showed higher counts of yeast cells than those of the producer A (table 1) Moreover, only 2 species were isolated from his yoghurt products The isolation of only 2 species referred to internal contamination of the manufacturing units Natural yoghurt of producer A contained lower counts of yeast cells and a wider spectrum of yeast species (table 1) *Saccharomyces cerevisiae* was only isolated from yoghurt of the producer A and represented one of the dominant species Generally, these results showed that the composition of yeast flora of yoghurt and total counts of yeast cells were dependent upon the manufacturing plants and contamination sources These observations were previously reported by several investigators (Katrandshiev, 1965, Tilbury et al , 1974, Dubois, 1980, Suriyarachchi & Fleet, 1981, Comi et al , 1982, Spillmann & Geiges, 1983, Hardy, 1987) Table 1 showed that not only higher counts of yeast cells but also wider spectrum of yeast species was detected at the surface of yoghurt packings than at its bottom *Kluyveromyces marxianus* represented the dominant species at the surface as well as at the bottom, whereas *Debaromyces hansenii* and *Saccharomyces cerevisiae* were only isolated from the surface These 3 species were the most common species recovered from yoghurt and their occurrence in yoghurt was reported by several investigators (Katrandzhiev, 1965, Dubois et al , 1980, Suriyarachchi & Fleet, 1981, Spillmann & Geiges, 1983, Hardy, 1987) Table 2 showed the ability of yeasts to grow at preservative temperature of yoghurt (5-6°C). It was clear from table 2 that yeasts could grow at the surface as well as at the bottom of yoghurt packings After the first day of manufacturing, one gram of yoghurt contained 1.4×10^4 and 5.4×10^4 yeast cells at the bottom and at the surface of yoghurt packing respectively (table 2), where as at the third day of manufacturing (consumption period allowed), the total counts of yeast cells reached 2.4×10^5 per g at the surface and 4.1×10^4 per gm at the bottom These results showed that yoghurt was heavily contaminated with yeasts and was not satisfactory for human consumption

II - Soft cheese:

Table 1 showed that soft cheese of producer G was heavily contaminated by yeasts The total counts of yeast cells ranged between 2.1×10^4 and 6.4×10^4 per g Its yeast cells counts was the highest count among the cheeses tested, where a limited number of yeast species was recorded These results indicate an internal contamination from manufacturing units The lowest number of yeast cell counts and the widest spectrum of yeast species was recorded in soft cheese of producer F Characteristic for cheese of producer E was the dominance of the red yeast species, *Sporobolomyces roseus* as compared to the common occurrence of *Kluyveromyces marxianus* and *Debaryomyces hansenii* in cheese of the producers F and G (table 1) Isolation of *Kluyveromyces marxianus* as a dominant species in soft cheese (Damietta) was reported by Fahmy & Youssef (1974) Dominance of *Kluyveromyces marxianus* and *Debaromyces hansenii* in other types of cheese was reported by several investigators (Stadhouder & Mulder, 1959, Lenoir & Auberger, 1966, Carini et al , 1977, Rosini, 1976, Vergeade et al , 1976, Deiana et al , 1977, Nakase & Komagata, 1977,

Table 1 Distribution of yeast species isolated from yoghurt, cheese, whey and dairy laboratory
Tableau 1 Répartition des espèces de levures isolées du yaourt, du petit lait et du laboratoire laitier.



A, B, and C = 3 different yoghurt producers D = fruit yoghurt of the producer C, E, F, and G = 3 different cheese producers
F and L = air, floor and equipment of dairy laboratory of produc. G respectively I = surface II = bottom R = cheese
S = whey L = number of samples in which yeast species was dominant K = Kluyveromyces Deb = Debaryomyces Rh = Rhodotorula
Sarch = Saccharomyces Sp = sporobolomyces T spora = Torulospora Tc = Trichosporon C = Candida

Table 2. Growth of yeasts in yoghurt at 5-6°C
Tableau 2. Croissance des levures dans le yaourt à 5-6°C

Incubation period (days)	Average numbers of yeast cells/g yoghurt	
	at the surface	at the bottom
1	5.4×10^4	1.4×10^4
2	7.6×10^4	1.9×10^4
3	2.4×10^5	4.1×10^4
4	3.3×10^5	7.4×10^4
5	4.7×10^5	1.2×10^5
6	6.1×10^5	2.5×10^5

Schmidt, 1978, Schmidt & Lenoir, 1978, 1980, Schmidt & Lambert, 1981, Haridy, 1987).

III - Whey:

Results in table 1 showed that higher counts of yeast cells and wider spectrum of yeast species were recorded in whey than in cheese It was clear from table 1 that the most common species of yeasts recovered from cheese were generally dominant in whey *Kluyveromyces marxianus, Debaromyces hansenii* and *Sporobolomyces roseus* were the dominant species *Rhodotorula mucilaginosa* and *Saccharomyces cerevisiae* were represented by considerable numbers of strains.

IV - Yeast flora of dairy laboratory:

In order to determine the effects of manufacturing practice on yeast flora of dairy products such as yoghurt and cheese, we chose dairy laboratory of the producer G Air, floor and equipment were screened for their contents of yeast flora Results in table 1 showed that air, floor and equipment represented a contamination sources of yoghurt and cheese by yeasts Yeast flora of the dairy laboratory, especially yeasts of the floor, was detected in yoghurt, cheese and whey This observation was also reported by Haridy (1987) Table 3 showed physiological and morphological properties of the isolated yeast species It was clear from table 3 that 252 isolated yeast strains were assigned to 10 species belonging to 9 genera *Kluyveromyces marxianus* was the most common species followed by *Debaromyces hansenii Rhodotorula mucilaginosa, Saccharomyces cerevisiae* and *Sporobolomyces roseus* were represented by considerable numbers of strains. Of particular note was the isolation of *Rhodotorula mucilaginosa, Trichosporon beigelii* and *Candida rugosa*, the human yeast pathogens, as well as *Candida steatolytica*, the erreger of mastitic udder of cow.

Table 3 Physiological and morphological properties of the isolated yeast species
Tableau 3 caractéristiques physiologiques et morphologiques des espèces de levures isolées

Yeast species	Total number of the isolates tested (252)	Fermentation								Assimilation											Growth at		Building of			
		glucose	galactose	maltose	sucrose	lactose	galactose	sorbose	ribose	xylose	arabinose	rhamnose	sucrose	maltose	lactose	raffinose	mannitol	gluconate	succinate	citrate	37°C	42°C	ascospores	pseudomycelium	true mycelium	pellicle
K. marxianus	104	100[a]	47	14	50	28	100	69	22	69	53	0	100	100	100	100	72	0	94	16	63	34	100	50	0	50
Deb. hansenii	58	83	46	13	50	0	100	100	79	00	75	33	100	100	92	100	100	92	100	100	50	0	100	0	0	25
Rh. mucilaginosa+	30	0	0	0	0	0	60	50	50	60	54	27	100	100	0	100	93	47	47	20	0	0	0	0	0	0
Sacch. cerevisiae	20	100	10	10	10	0	100	0	0	0	0	0	100	100	0	30	10	0	50	0	80	10	100	40	0	40
Sp. roseus	20	0	0	0	0	0	50	0	0	0	0	0	100	100	0	100	50	0	0	0	0	0	0	0	0	0
Cr. albidus	6	0	0	0	0	0	100	100	100	00	100	67	100	100	100	100	100	100	100	67	0	0	0	0	0	0
T'spora delbrueckii+	6	100	67	33	67	0	100	67	0	33	0	0	100	100	0	100	100	67	67	0	33	0	100	50	0	50
Tr. beigelii+	4	0	0	0	0	0	100	100	100	100	100	100	100	100	100	100	100	0	0	0	0	0	0	100	100	100
C. rugosa+	2	0	0	0	0	0	100	0	0	0	0	0	0	0	0	0	100	0	0	0	100	0	0	100	0	100
C. steatolytica$	2	100	0	0	100	100	100	100	100	00	0	100	100	100	100	100	100	100	100	100	100	0	0	100	00	100

a = Percentage of positive reactions of strains + = Human yeast pathogens $ = Erreger of mastitic udder of cow

C. = Candida C. = Cryptococcus Deb. = Debaryomyces Rh. = Rhodotorula Sacch. = Saccharomyces T'spora = Torulaspora

Tr. = Trichosporon Sp. = Sporobolomyces.

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